Evaluation of a New Monoclonal LGR5 Antibody (Clone UMAB212) for Multiple Immunoassay Applications

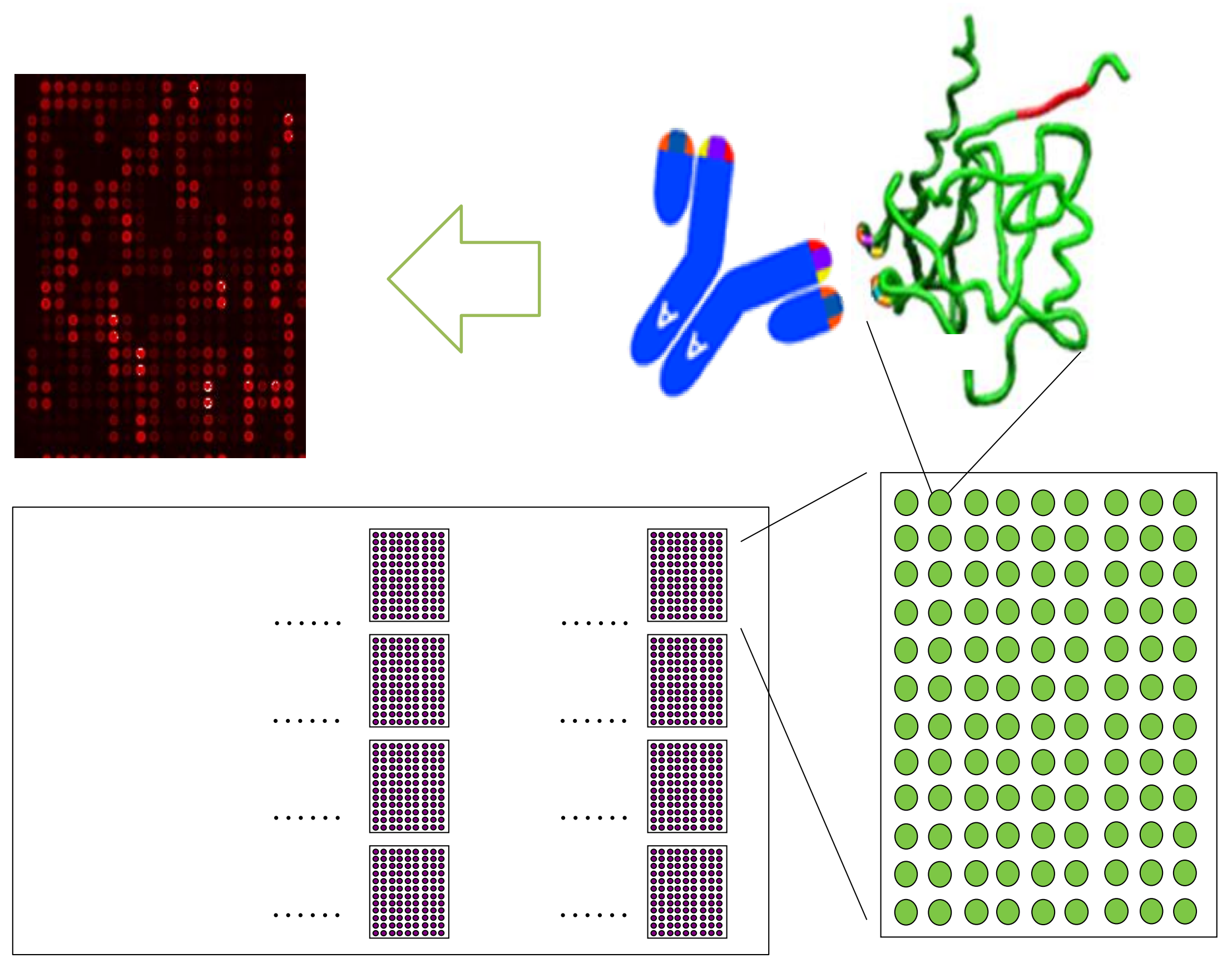
Wei Fu, Rachel Gonzalez, Kehu Yuan, Caiwei Chen, Haitao Wei, H. Emily Lu, Qi Ren, Evelin Logis, Sean Kelly, Wei-Wu He and Donghui Ma
OriGene Technologies, 9620 Medical Center Dr., Suite 200, Rockville, MD 20850

Introduction

Cancer stem cells are a subpopulation of cancer cells responsible for cancer initiation, development and metastasis. A number of studies demonstrated that Leucine-rich repeat containing G-protein-coupled receptor 5 (LGR5) can drive cancer development through triggering canonical Wnt signaling and its downstream gene expression. LGR5 is an important biomarker specifically expressed on colon cancer stem cells. In this study, we have successfully developed an LGR5 antibody with high specificity to detect endogenous LGR5 expression in different immunoassay application.

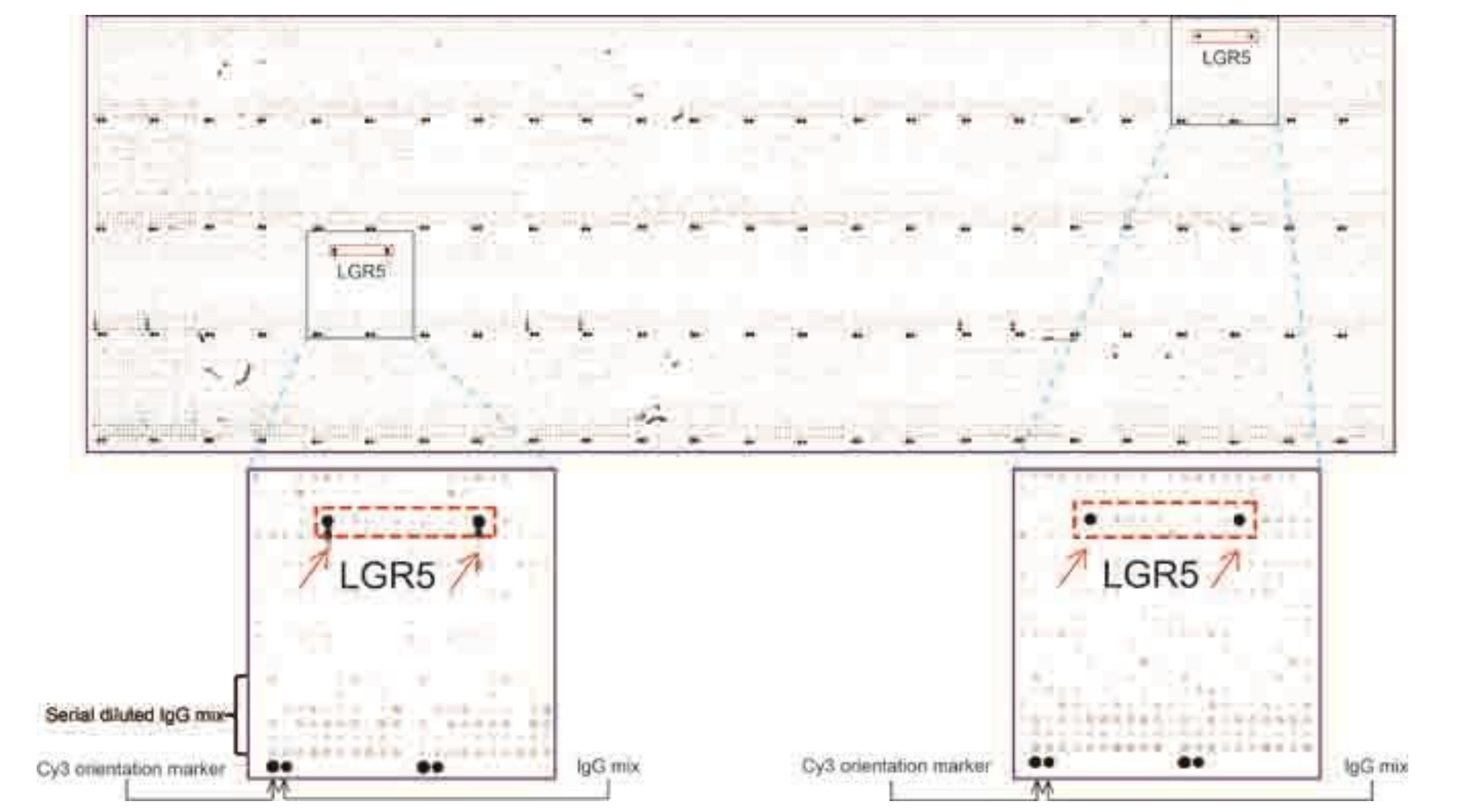
By screening multiple anti-LGR5 hybridoma clones, antibody generated by clone UMAB212 was proven to be highly specific on our high density protein microarray chip assay. Here our experimental data demonstrated that clone UMAB212 recognizes not only human LGR5 protein but also mouse LGR5 protein in both western blot and flow cytometry applications. No cross–reactivity was observed with the other two LGR family members, LGR4 and LGR6. Furthermore, this antibody also works great on immunohistochemistry application on FFPE tissue blocks. In summary, UMAB212 is great tool for us to study LGR5 protein in different immunoassay setting and it could also be a potential cancer diagnostic reagent.

OriGene Protein Microarray Chip for antibody specificity identification



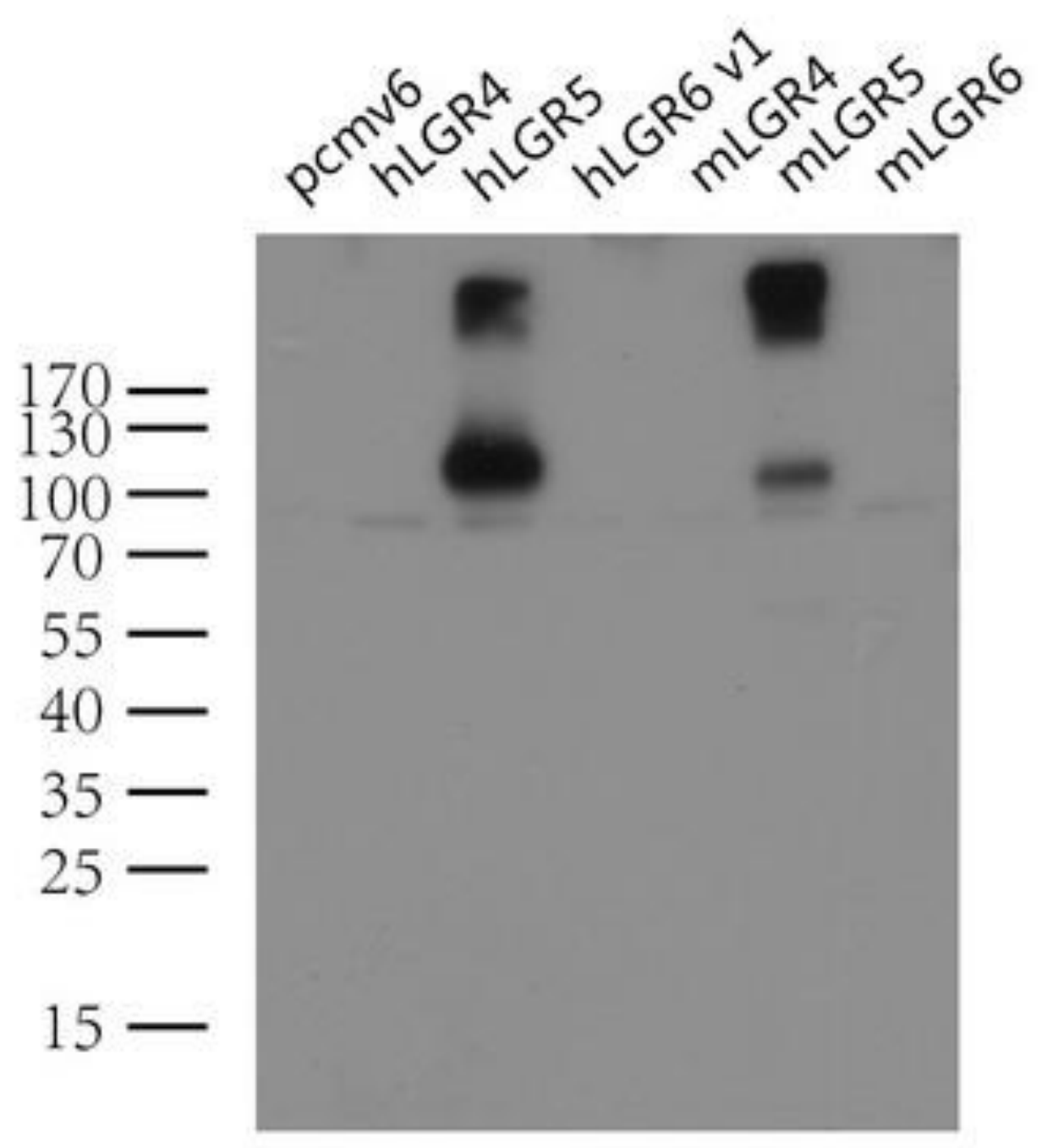
OriGene developed a high-density protein microarray to test antibody-antigen interaction in a high-throughput manner. (All of ultraMAB® are identified by OriGene high density protein microarray chip for specificity. http://origene.com/UltraMAB/ with specificity service provided at http://origene.com/UltraMABService/)

The newly developed LGR5 monoclonal antibody (UMAB212) is highly specific

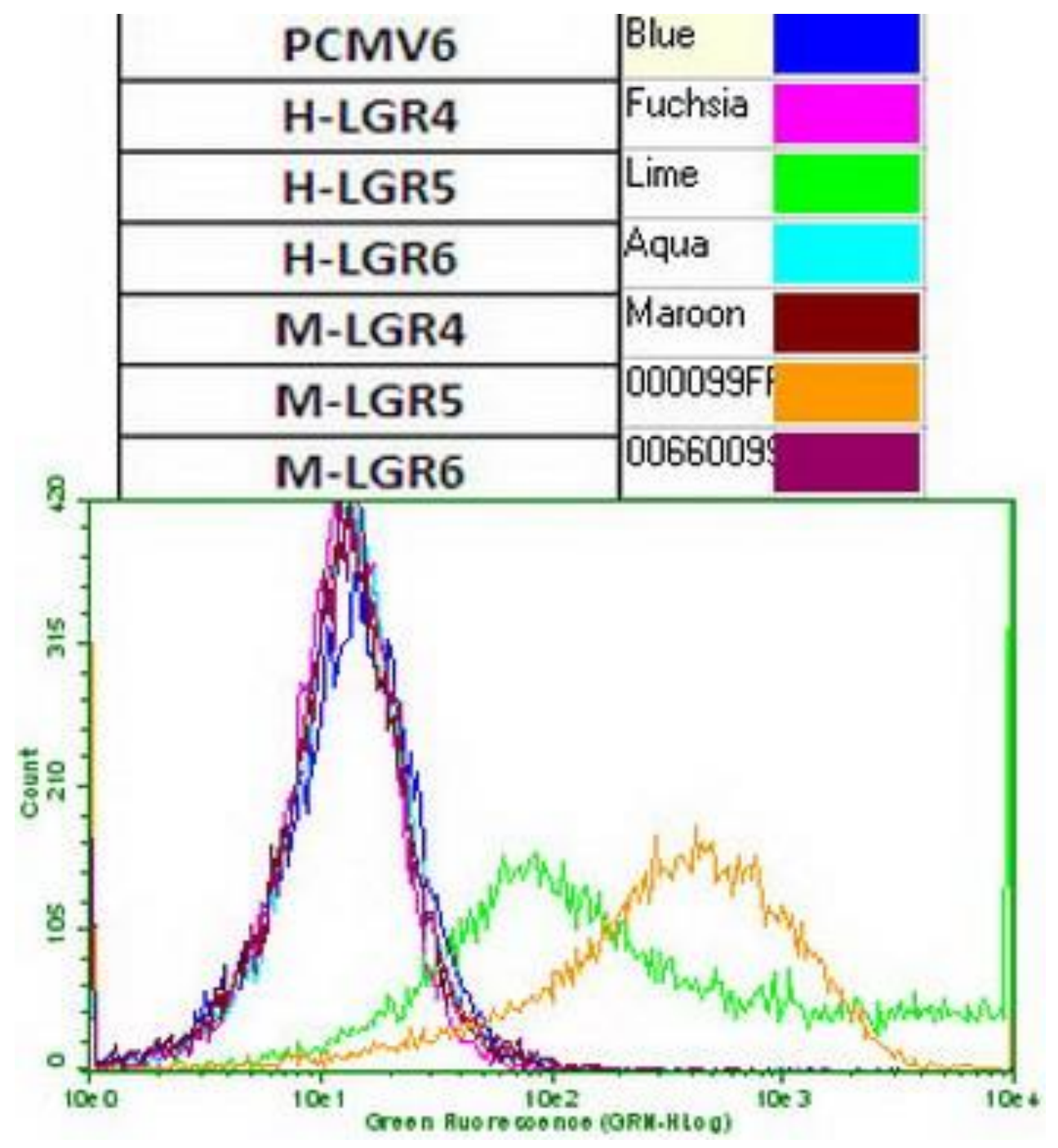


UltraMAB anti-LGR5 mouse monoclonal antibody (UMAB212) on high density protein microarray chip. The positive reactive proteins are highlighted with two red arrows in the enlarged sub-arrays. LGR5 mouse monoclonal antibody (UMAB212) does not react with any other proteins on the array chip.

UMAB212 recognizes both human and mouse LGR5, but does not cross-react with LGR4 and LGR6

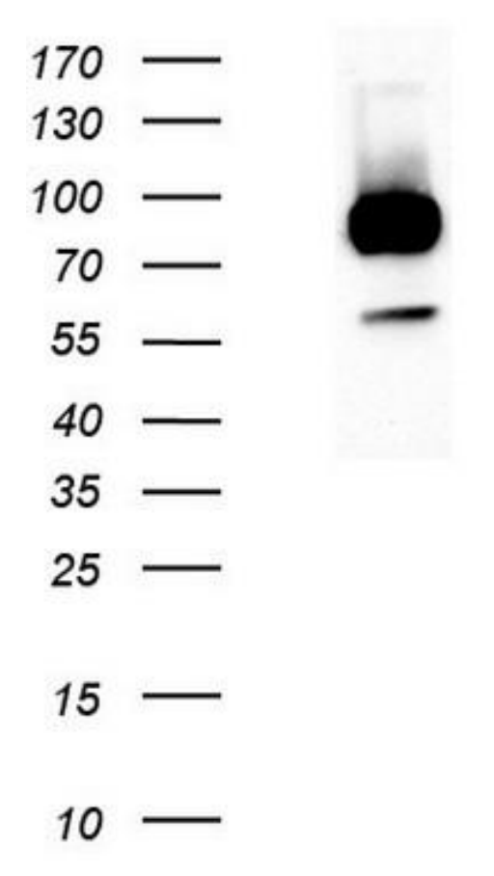


Western blot analysis of extracts from seven different cDNA transiently transfected HEK293T cell lysates by using UMAB212

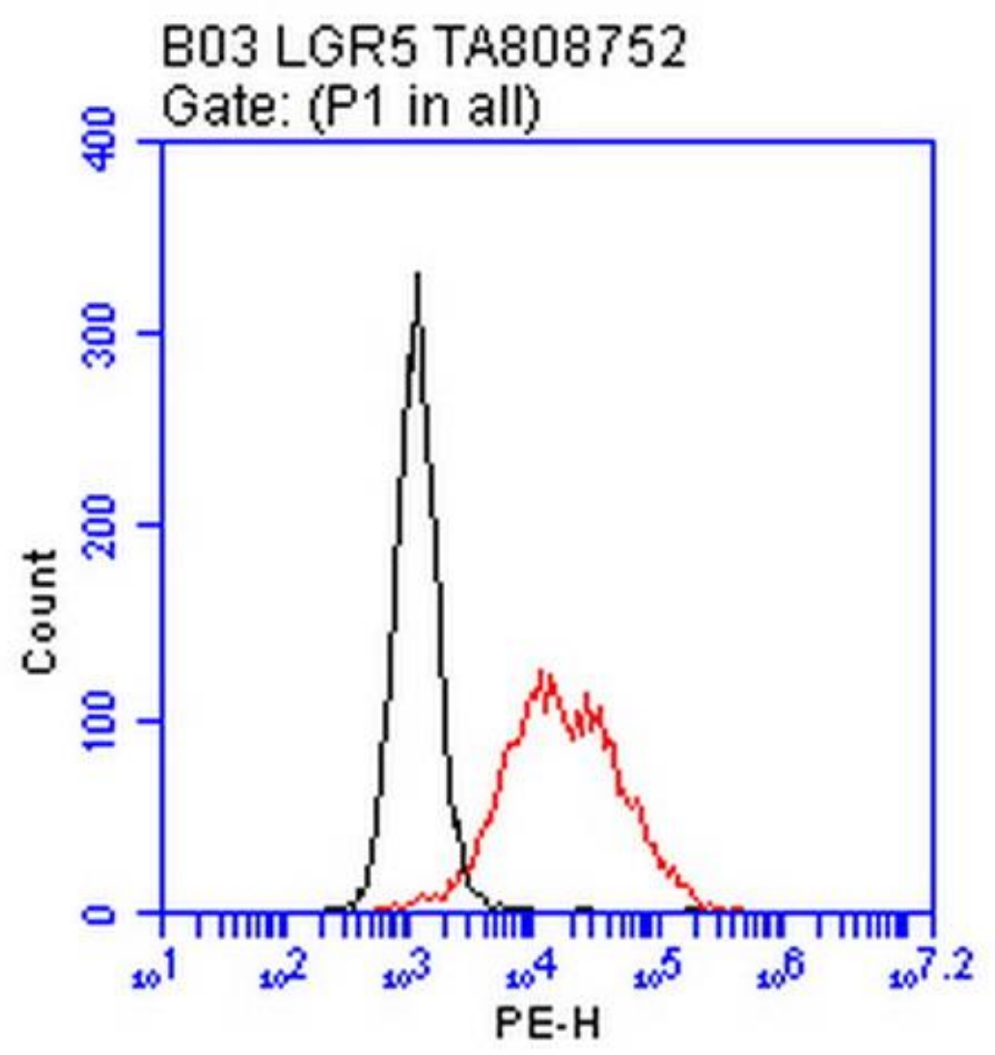


Flow cytometric Analysis of HEK293T cells transiently transfected with human or mouse LGR4, human or mouse LGR5, human or mouse LGR6, or control vector pCMV6-Entry using UMAB212

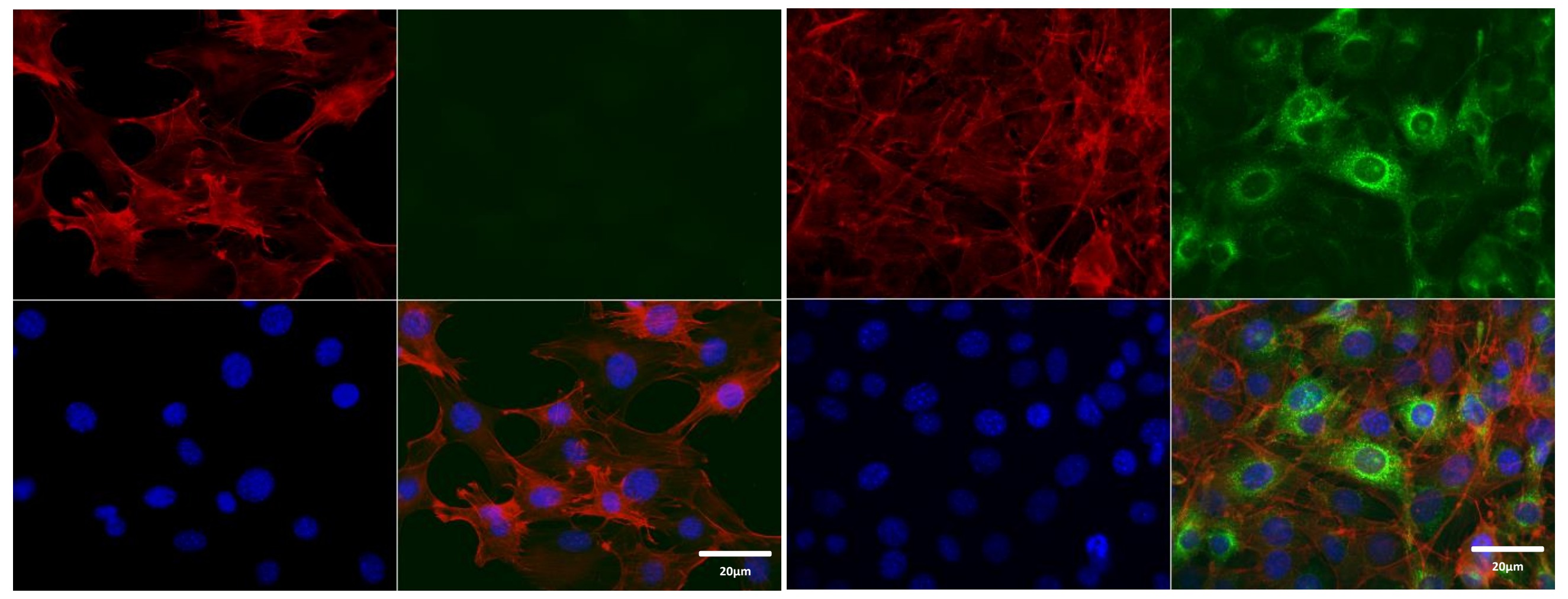
UMAB212 for multiple applications (WB, Flow Cytometry, and Immunofluorescence)



NIH-3T3 (Left lane) or stable expressed LGR5-3T3 cell lysate (right lane) were immunoblotted with anti-LGR5 UMAB212

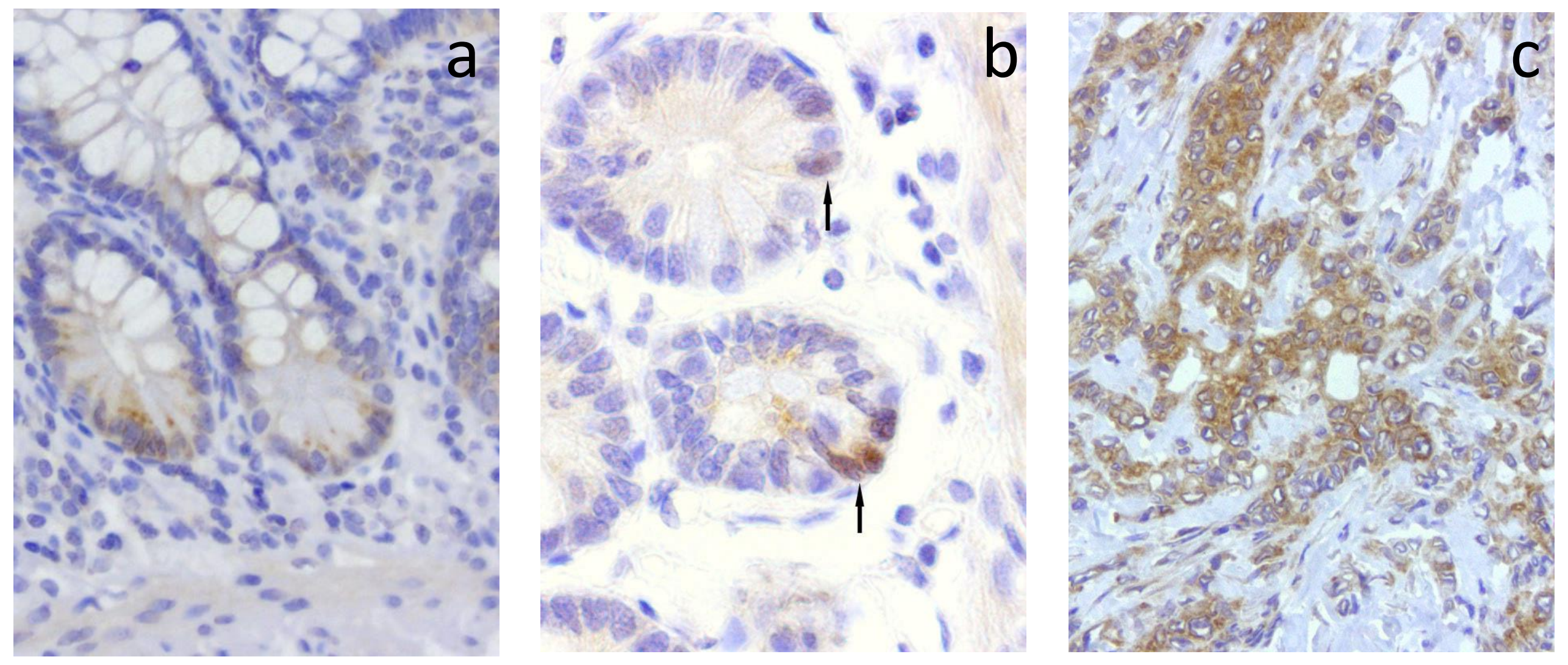


Flow cytometric analysis of the stable expression of LGR5 in NIH3T3 cells (alive) using anti-LGR5 antibody (UMAB212) (Red) vs negative CTL (Black)



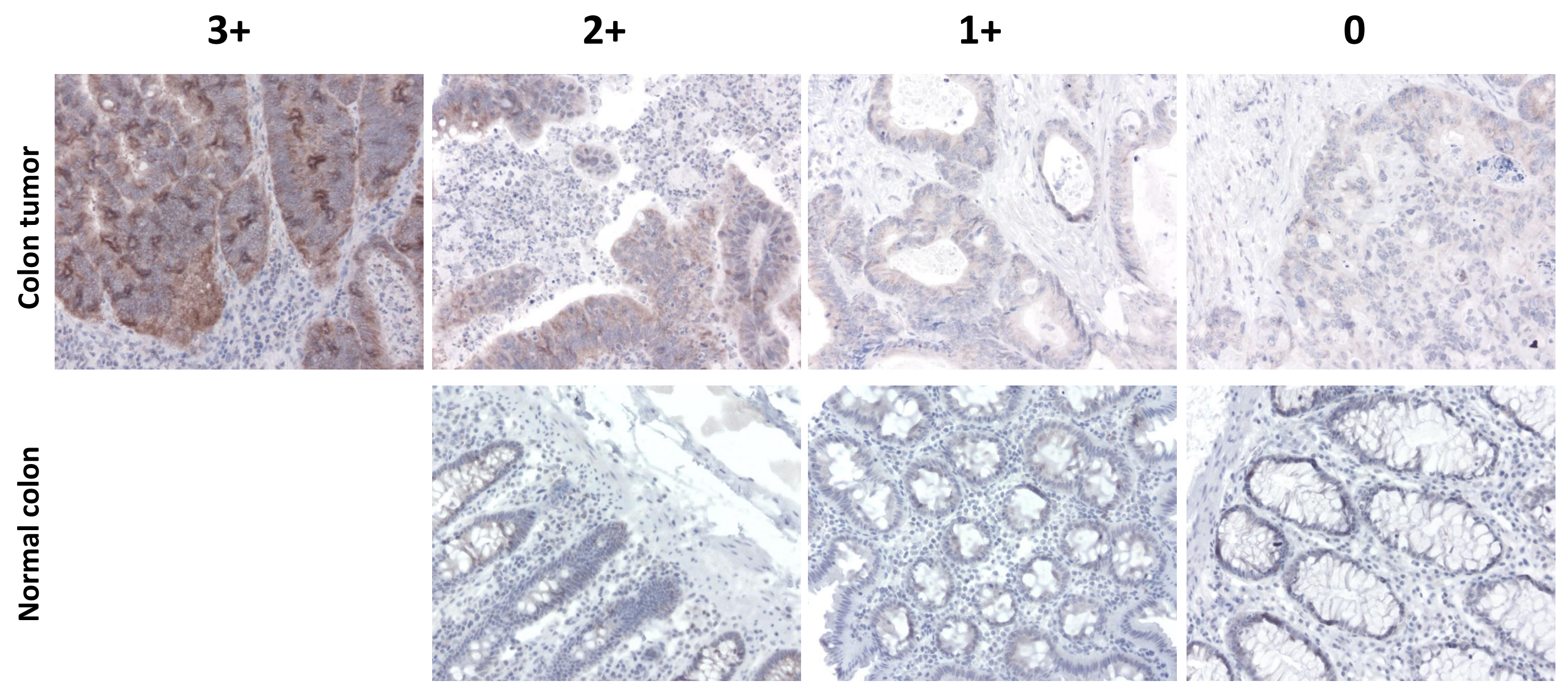
Immunofluorescence staining of 3T3 cells (left panel) or LGR5 stable expression 3T3 cells (right panel) using LGR5 antibody (UMAB212, green), Phalloidin (red) and DAPI (blue)

UMAB212 for companion diagnosis on human FFPE gastrointestinal tissues



Immunohistochemistry staining of LGR5 antibody (UM<AB212) on paraffin-embedded human small intestine tissue (a, b) and gastric cancer (c). The arrows point to weak and strong staining of small intestine stem cells

UMAB212 IHC staining and analysis on human colon tissue microarray



Score	3+	2+	1+	0	Subtotal
colon tumor	5	18	8	6	37
colon tumor	14%	49%	22%	16%	100%
normal colon	0	1	1	5	7
normal colon	0%	14%	14%	72%	100%

Immunohistochemistry staining of LGR5 antibody (UMAB212) on FFPE human colon tissue array (top panel, 37 from human colon cancer; bottom panel, 7 from normal colon tissues)

Conclusions

1. We developed a highly specific LGR5 UltraMAB® with our high density protein microarray chip technology.
2. Clone UMAB212 does not cross-react with other LGR family members such as LGR4 or LGR6.
3. Clone UMAB212 recognizes both human and mouse LGR5.
4. Clone UMAB212 can be used for multiple immunoassays (Western blot, Flow, immunofluorescence and immunohistochemistry).
5. Clone UMAB212 may be considered as therapeutic monoclonal antibody.

References

1. Haegebarth A and Clevers H. Wnt signal, lgr5 and stem cells in the intestine and skin. The American Journal of Pathology, 2009, 174: 715
2. Ma D, et al. Using protein microarray technology to screen anti-ERCC1 monoclonal antibodies for specificity and applications in pathology. BMC Biotechnology, 2012, 12: 88
3. Barker N, et al. Identification of stem cells in small intestine and colon by marker gene LGR5. Nature, 2007, 449: 003
4. Marx V, Finding the right antibody for the job. Nature Methods, 2013, 10, 703
5. Jang BG, Distribution of LGR5+ cells and associated implications during the early stage of gastric tumorigenesis. PLoS One, 2013, 8, e82390